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Corresponding Author: Miss Elenor Morgenroth, M.Sc., B.Sc.

Corresponding Author's Institution: University of Roehampton

First Author: Elenor Morgenroth, M.Sc., B.Sc.

Order of Authors: Elenor Morgenroth, M.Sc., B.Sc.; Natasza Orlov, PhD; David J Lythgoe, PhD; James M Stone, PhD; Holly Barker, MSc; James Munro, PhD; Michael Eysenck, PhD; Paul Allen, PhD

Abstract: Trait anxiety can affect cognitive control resulting in ineffective and/or inefficient task performance. Moreover, previous functional Magnetic Resonance Imaging (fMRI) studies have reported altered dorsolateral prefrontal cortex (DLPFC) activity in anxious cohorts, particularly when executive control is required. Recently, it has been demonstrated that cortical glutamate levels can predict both functional activation during cognitive control, and anxiety levels. In the present study we sought to investigate the relationship between trait anxiety, prefrontal glutamate levels and functional activation in DLPFC during a cognitive control task. Thirty-nine participants assigned to either low trait anxiety (LTA) or high trait anxiety (HTA) groups underwent 1H-Magnetic Resonance Spectroscopy (1H-MRS) to measure levels of resting glutamate in the prefrontal cortex (PFC). Participants also completed fMRI during a Stroop task comprising congruent and incongruent colour word trials. The HTA group showed reduced task performance relative to the LTA group. In the LTA group, there was a positive association between PFC Glu levels and DLPFC activation during incongruent trials. This association was absent in the HTA group. Individual differences in trait anxiety affect the relationship between PFC glutamate levels and DLPFC activation, possibly contributing to ineffective task performance when cognitive control is required.

*Title page

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AUTHORS: Elenor Morgenroth MSc [1], Natasza Orlov PhD [1, 2], David J Lythgoe PhD [3], James M Stone PhD [3], Holly Barker MSc [1], James Munro PhD [1,4], Michael Eysenck PhD [1, 5], Paul Allen PhD [1, 2, 6]

Affiliations

1. Department of Psychology, University of Roehampton, London, United Kingdom (UK)
2. Department of Psychosis Studies, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK
3. Centre for Neuroimaging Sciences, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK
4. Department of Psychology, Edinburgh Napier University, Edinburgh, UK
5. Department of Psychology, Royal Holloway University of London, London UK
6. Combined Universities Brain Imaging Centre, London, UK

Corresponding author: Elenor Morgenroth

Department of Psychology, University of Roehampton, Whitelands College, Hollybourne Avenue, London SW15 4JD

Email: morgenre@roehampton.ac.uk

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13th February 2019

Dear Professor Kirsch,

We thank you for inviting us to resubmit our manuscript. We feel that throughout this process the Reviewers' comments and suggestions have helped to greatly improve the manuscript. We have now attempted to address Reviewer 1's further comments (see below). We have particularly focused on Reviewer 1's request for us to clarify the issues around power and null findings. In accordance with this request we have made a further amendment to the Discussion section. We hope that this and the other minor changes are satisfactory and that we can now publish our manuscript in Cortex.

Yours sincerely

Elenor Morgenroth

Reviewer #1:
I found the manuscript to be very much improved, in particular with regard to the theoretical explanation of the research topic and the embedding into the existing literature. While I still have reservations regarding the statistical power of the study, I recommend this study for publication because of the novelty and relevance of the research question. I have some further comments I would like to ask the authors to consider. The one point I would suggest as a condition for publication is the still more explicit discussion of the power / null finding issue.

You report Bayes Factors for some analyses now. Would you please interpret these BFs with reference to some literature about the meaning / significance of these Factors? Do I understand correctly, that all the BFs you report are < 1? Then they do not really support either the null or the alternative hypotheses? In my opinion, you must discuss this in your paper. I would say, everywhere where you mention a BF.

We thank the reviewer for this comment and have added more detail regarding the interpretation of the Bayes Factors in the Methods section on Page 7

A BF_{10} > 3 is considered evidence for H_1 whereas a BF_{10} < 1/3 is considered evidence for H_0 [39].

And also in the discussion section on the power limitations of your study. I'd say your null findings can be interpreted in the sense that there are probably no large effects (which you would have had power to detect).

But you do not provide proof - or even strong evidence - that there is actually no effect. I find the subject of the study very interesting and recommend it for publication. But I strongly suggest that you make this specific limitation even more explicit than in the revision so far. Explicitly discuss the meaning of the BF, please. And make explicit, that you had sufficient power to detect effects of a size that have been reported before. But still, there where pretty large effects (approx. .6 - .9). I'd say these are not the smallest effects of interest in research on neural correlates of trait anxiety. Explicitly state that there may be smaller effects you did not have power to detect with the current sample.

We agree with the reviewer that the power and null effects issue does need further discussion and clarification. We have therefore attempted to expand on this issue in the Discussion section on Page 17 with a more thorough consideration of the null findings in light of the study's power

First, we report null findings that raise issues regarding the power of the study. Our power calculations (Supplementary Material) suggest that the study was sufficiently powered to detect medium to large effect sizes (.50 - .90), that have been reported previously by studies investigating the associations between DLPFC activity [5], cortical glutamate levels [29] and trait anxiety. Clearly however, our study was not sufficiently powered to detect smaller effects sizes. This is important because previous studies examining the effects of trait anxiety on neurotransmitter levels for example have reported smaller effects sizes e.g. [28]). Furthermore, Bayes Factors did not give a strong indication for either the null or the experimental hypothesis with regard to the relationship between trait anxiety and PFC Glu levels.

Thus, the null findings reported here need to be interpreted with caution in as much as the study sample only provides sufficient power to detect larger effects. We cannot discount the possibility that the significant relationships between trait anxiety, DLPFC activity and/or cortical glutamate levels might be observed in a study powered to detect smaller effects sizes.

I am still wondering, why you did not see a classic Stroop effect in your task as this is usually so very robust. Also, mean RTs between 900 und 1100 ms seem to be really high for a Stroop task. Could you please discuss that with reference to literature on the Stroop task? Could there have been some kind of ceiling effect in

Stroop RTs that prevent you from seeing the classic differences between incongruent (IC) and congruent (CO) trials. And if so, any ideas why? Is there something in your version of the task that makes IC and CO more similar than usually?

We used a single-handed 4-finger button box rather than a 2-hand, 2-finger response system, as used in previous studies (e.g. Basten et al. 2011). We cannot be sure how this affected our behavioural results during the Stroop task but it is possible that this response set-up may have led to relatively high reaction times compared to other studies. Reaction times were distributed symmetrically and the high mean and lack of Stroop effect does not seem to be driven by outliers. We addressed this in the Discussion on Page 15.

It is unclear why this reaction time pattern was observed but it may have been due to a speed accuracy trade-off, trial/task pacing [54], or because the version of the task used in the present study used a single handed four-finger response system accounting for the relatively high reaction times observed in both congruent and incongruent task conditions. .

I would still expect more and stronger differences in BOLD for the two conditions even at .05 FWE, and some effect in the default mode network - after all, you are studying 39 subjects here.

Throughout our manuscript we have reported effects at a strict peak-level corrected threshold ($p < .05$ FWE). At a cluster corrected level ($p = .05$ FWE) there is more extensive activation in the anterior cingulate extending to the supplementary motor area and also the bilateral DLPFC and superior parietal lobes, caudate/putamen and cerebellum. Taking into account the strict threshold our results do compare to previous research using the Stroop task in fMRI (e.g. Zysett et al. 2007).

For the association between anxiety and BOLD anti in DLPFC you write: "The effect of trait anxiety (STAI trait scores) on DLPFC activation was non-significant in bilateral DLPFC ROI during Incongruent > Congruent trials." Could you quantify what non-significant means here? As you are working with a priori defined ROIs, you could extract beta estimates from these ROIs and calculate the corrections offline. Also the association between anxiety and GLU could be calculated as a correlation offline to provide an estimate of the effect size of association when considering both as continuous variables. This would be useful information for the reader.

We have added the requested correlation between Trait Anxiety and PFC Glu Corr levels to the Results section.

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The correlation between Trait anxiety scores and PFC Glu Corr levels was also non-significant ($r = .25$ $p = .121$).

As requested, we have also calculated the correlation between beta estimates and trait anxiety scores offline, which was r = -.04, p = .809 for the right DLPFC ROI and r = .03, p = .880 for the left DLPFC ROI. We did not include these in the manuscript as we explicitly do not want to present any secondary and potentially non-independent testing with extracted beta values from fMRI analyses (Vul et al. 2009; Eklund et al. 2016).

I found the theoretical explanations to be very much improved in general. One point that is still not clear to me is why "... the absence of this relationship between resting Glu levels and DLPFC activity in the HTA group may result in ineffective task performance". How is the association of GLU and BOLD important or necessary for successful task performance - or how could it be theoretically.

We have previously tried to address this issue in the Introduction and Discussion of the revised manuscript. Essentially, the precise relationship between resting PFC Glu levels and neural activity during cognitive control are not fully understood. However, we have tried to develop our theoretical argument by making suggestions for future work using task related MRS that could provide greater insight into underlying processes. We have expanded upon this in the Discussion on Page 18.

Page 18
Thus, future work could measure task-related differences in Glu levels to obtain a more accurate and dynamic insight into the neural basis of cognitive processes [67]; combined fMRI and MRS i.e. scan data collected simultaneously is a promising method to better understand the relationship between BOLD and neurotransmitter levels in the context of task processing [68].

ABSTRACT

Trait anxiety can affect cognitive control resulting in ineffective and/or inefficient task performance. Moreover, previous functional Magnetic Resonance Imaging (fMRI) studies have reported altered dorsolateral prefrontal cortex (DLPFC) activity in anxious cohorts, particularly when executive control is required. Recently, it has been demonstrated that cortical glutamate levels can predict both functional activation during cognitive control, and anxiety levels. In the present study we sought to investigate the relationship between trait anxiety, prefrontal glutamate levels and functional activation in DLPFC during a cognitive control task**.** Thirty-nine participants assigned to either low trait anxiety (LTA) or high trait anxiety (HTA) groups underwent ^{1}H-Magnetic Resonance Spectroscopy (^{1}H-MRS) to measure levels of resting glutamate in the prefrontal cortex (PFC). Participants also completed fMRI during a Stroop task comprising congruent and incongruent colour word trials. The HTA group showed reduced task performance relative to the LTA group. In the LTA group, there was a positive association between PFC Glu levels and DLPFC activation during incongruent trials. This association was absent in the HTA group. Individual differences in trait anxiety affect the relationship between PFC glutamate levels and DLPFC activation, possibly contributing to ineffective task performance when cognitive control is required.

1. INTRODUCTION

Trait anxiety is a normally distributed personality dimension and a risk factor for anxiety and depressive disorders [1, 2] characterised by intrusive thoughts, worry and difficulty in disengaging from negative material [3]. Trait anxiety has been found to be associated with functional consequences including increased distractibility and attention problems [4-6]. Indeed, the effects of trait anxiety on cognitive function have long been recognised [7] and are accounted for by attentional control theory (ACT; [6, 8]).

ACT proposes that anxiety competes for attentional resources and impairs cognitive control when executive processes are required i.e., updating, set shifting and inhibiting irrelevant or distracting information. Consequently, anxiety can impair task performance i.e. *performance effectiveness* when executive control is required. Further, ACT predicts that, even when performance effectiveness is maintained, anxiety can reduce *processing efficiency* (the quality of performance relative to use of processing or cognitive resources). In line with this prediction, functional Magnetic Resonance Imaging (fMRI) studies report increased prefrontal cortex (PFC) activation in people with high trait anxiety without concomitant improvements in performance effectiveness (i.e. processing inefficiency; [9-11]). The PFC along with the lateral parietal cortices i.e. the fronto-parietal network (FPN), are known to be important for cognitive control [12, 13] and support 'top-down' attention by maintaining attentional sets [12, 14, 15]. In particular the dorsolateral prefrontal cortex (DLPFC), comprising the middle and superior frontal gyri, has a central role in top-down cognitive control [16] and has been shown to have altered activation in response to tasks that require cognitive control in people with high trait anxiety (e.g. [5, 9-11]).

Despite these recent advances in our understanding of the neural mechanism involved in cognitive control, little is known about its neurochemistry and how this may be affected by

individual differences in trait anxiety. Glutamate (Glu) is an excitatory neurotransmitter and its importance in cognitive control has been highlighted in animal models [17, 18]. In humans, Anticevic and colleagues [19] showed that administration of ketamine, an N-methyl-D-aspartate glutamate receptor (NMDAR) antagonist, disrupts activity in FPN regions and subsequent performance during a working memory task, highlighting the role that Glu plays in cognitive control. Combining functional Magnetic Resonance Imaging (fMRI) and ^{1}H-Magnetic Resonance Spectroscopy (^{1}H-MRS), Falkenberg and colleagues [20] demonstrated that the magnitude of the blood-oxygen level-dependent (BOLD) response to a task requiring cognitive control was predicted by anterior cingulate resting state Glu levels. Moreover, individual variability in resting Glu levels was related to how the brain implements cognitive control [20].

These findings are important because Glu functioning is altered in some psychiatric disorders associated with cognitive control impairments [21] and pharmacologically induced reductions in Glu levels have been found to alter the BOLD response during cognitive control tasks [22, 23]. However, whilst in vivo ^{1}H-MRS studies investigating the neurobiology of anxiety have focused on populations with diagnosed disorders (e.g. [24-27]), ^{1}H-MRS studies in non-clinical populations in which trait anxiety is assessed as a personality dimension are relatively few in number. The first study using ^{1}H-MRS to examine metabolite levels in relation to trait anxiety reported increased PFC N-Acetyl aspartate (NAA) in participants with high trait anxiety levels but found no differences in Glu levels between high and low trait anxiety participants [28]. More recently, Modi and colleagues [29] reported that cortical Glu and combined Glu and glutamine levels (measured with ^{1}H-MRS in the anterior cingulate) were increased in participants with high relative to low trait anxiety scores and predictive of trait anxiety levels across their study cohort. Pharmacologically induced anxiety has also been reported to increase cortical Glu levels [30].

Together, the studies discussed here indicate that trait anxiety can affect both DLPFC activity during cognitive control and PFC Glu levels. Whilst it has already been established that resting cortical Glu levels are important for the way the brain implements cognitive control [19, 20], to date, no studies have measured resting cortical Glu levels and DLPFC activity during a cognitive control task and examined how these are related to individual differences in trait anxiety levels. This is important because it is possible that the effects of trait anxiety on DLPFC activity (and cognitive control) are influenced by cortical Glu levels. Although the precise relationship between resting PFC Glu levels and neural activity is not fully understood, a number of studies have shown that levels of resting Glu measured with ^{1}H-MRS are related to the BOLD signal and electrophysiology measures during cognitive tasks [20, 31-33] and possibly mediated via NMDAR [19].

The aim of the present study was to investigate the relationship between trait anxiety, PFC Glu levels (using ^{1}H-MRS) and activity in DLPFC during a cognitive control task. In accordance with the predictions of ACT and findings from previous fMRI studies, we hypothesised that levels of trait anxiety would be positively associated with DLPFC activity during a cognitive control task (indicative of processing inefficiency). Based on the findings outlined above, we then tested if participants with high levels of trait anxiety had elevated levels of PFC Glu relative to a low trait anxiety group. Finally, we explored how the association between resting PFC Glu levels and DLPFC activity during cognitive control was affected by individual differences in trait anxiety levels. No part of the study procedures or analyses was pre-registered in a time-stamped, institutional registry prior to the research being conducted.

2. METHODS

We report how we determined our sample size (see Supplementary Materials). No data were excluded and inclusion/exclusion criteria are reported below. Inclusion/exclusion criteria were established prior to data analysis as were all manipulations, and all measures in the study. The raw data and materials to replicate this study or any analysis are available at Open Science Framework (DOI 10.17605/OSF.IO/PXK8Z).

2.1.Participants and assessments

Thirty-nine participants performed a colour-word Stroop task [34] while functional magnetic resonance imaging and ^{1}*H-MRS* data were acquired. Participants (27 female) ranged from 18-37 years of age (M = 22.05 years, SD = 4.62). There were 35 right handed and four left handed participants, as assessed by the Annett Hand Preference Questionnaire [35]. Participants were recruited from the University of Roehampton, Royal Holloway University of London and from the general public. Participants had no prior neurological or medical illness and were not using medication for anxiety or depression. The University of Roehampton Ethics Committee gave ethical approval and all participants gave written informed consent prior to taking part in the study. IQ was estimated using the Wide Range Achievement Test (WRAT-R) Reading Level 2 [36]; M = 109.15 (SD = 10.24, Range 86-131) to control for potential effects of IQ on task performance and task-related BOLD signal. Alcohol consumption and recreational cannabis use were assessed for all participants using a categorical scale (ranging from no-use to regular use). The majority of participants indicated that they used alcohol on a moderate basis and that they used cannabis never or only experimentally (see Table s1).

To assess trait anxiety, participants completed the State Trait Anxiety Inventory (STAI) [37]. In all participants the mean score for trait anxiety was 41.33 (SD = 11.07, Range 22-78) and

33.2 (SD=10.01, Range = 20-70) for state anxiety. This distribution of STAI trait scores is slightly higher than published norms (i.e. M = 36, SD = 10) [37] but comparable to scores reported by a previous study examining effects of trait anxiety on DLPFC activation (i.e. M = 43 SD = 11) [5].

A median-split of STAI trait scores was used to establish low trait anxious (LTA; n = 19) and high trait anxious (HTA; n = 20) groups (see results), this dichotomization was performed to achieve greater interpretability of the results. Confirmatory analysis of behavioural and MRI data using STAI trait scores as a continuous variable are reported in the Supplementary Materials.

2.2.Experimental Task

Participants performed a colour-word Stroop task adapted for MRI and used previously [38]. The task was programmed and presented with Microsoft Visual Basic. Participants responded with one of four fingers of their right hand to the font colour of the word presented (Red, Yellow, Blue or Green). Participants were instructed to respond as quickly and as accurately as possible while reaction times (RT) and error rates (ER) were recorded. The task consisted of a total of 100 trials, 33 congruent trials in which the font colour and meaning of the word matched, 33 incongruent trials in which the font colour and meaning of the word did not match and 34 fixation periods in which the participants saw a fixation cross. Trials were presented in a pseudo-randomized order within one functional run lasting 10 minutes. Each trial (including fixation cross trials) was presented in the middle of the screen and took 6000 ms including a period of 1300 ms before trial onset in which a blank dark grey screen was displayed. Participants then viewed a visual stimuli (i.e. congruent word, incongruent word, or fixation cross) that was presented for 700 ms. Thus participants were allowed 4700 ms from stimulus onset (700 ms during trial presentation plus 4000 ms response period) to

respond i.e. responses were registered from the onset of each stimulus trial. After a response was registered the trial continued until the end of this period. No response was required in fixation cross trials.

2.3.Statistical Analysis and Power

IBM® SPSS Statistics Version 22 was used for the analysis of task and questionnaire data. Questionnaire and task data were considered normally distributed. A multifactorial repeated measures ANOVA with the dependent variables RT and ER in the two conditions of the Stroop task (Congruent, Incongruent) was performed. Trait anxiety group was included as a between subjects factor. A statistical significance threshold of $p < .05$ was applied throughout. To test if analyses were sufficiently powered we used G*Power (https://download.cnet.com/G-Power/3000-2054_4-10647044.html). Power calculations are reported in Supplementary Material. Furthermore, we used statistical software program JASP (JASP Team, 2016; jasp-stats.org) to compute Bayes Factor (BF_{10}) to quantify the relative likelihood of the model tested to the null hypothesis. A $BF_{10} > 3$ is considered evidence for H_1 where as a $BF_{10} < 1/3$ is considered evidence for H_0 [39]. LTA and HTA groups were compared on STAI trait and state scores, IQ estimate and age using independent samples t-tests. The groups were also compared on their alcohol consumption and cannabis use using Mann-Whitney U tests for ordinal data.

2.4.MRI Acquisition

All MRI scans were acquired on a 3T Siemens Magnetom TIM Trio scanner using a 32-channel head coil. Structural T1 weighted Magnetization Prepared Rapid Acquisition Gradient Echo (MP RAGE) images were acquired with a spatial resolution of 1 mm × 1 mm × 1 mm, in plane resolution of 256 × 256 × 176 slices and scanning time of approximately 5 minutes. Functional images were acquired using a full-brain, anterior-to-posterior, T2*

weighted, BOLD-sensitive gradient echo planar sequence with the following parameters: TR/TE/flip angle = 2 s/40 ms/70°, field of view 192 mm × 192 mm and slice thickness of 5 mm giving a voxel size of 3 mm × 3 mm × 5 mm and whole brain coverage of 28 interleaved slices. Three hundred volumes were collected during the event related functional run.

2.5.^{1}H-MRS data acquisition and analysis

^{1}H-MRS in vivo spectra were acquired from a 20 × 20 × 20 mm voxel located in the right medial PFC during rest. A voxel in the right PFC was chosen as previous fMRI studies report effects of anxiety in the right PFC [9, 10]. A medial position was chosen as lateral voxels can be harder to place due to tissue boundaries. The voxel was positioned manually by reference to an axial T1- weighted gradient echo image (Figure 3B). Spectra were acquired using SPin ECho full Intensity-Acquired Localized spectroscopy (SPECIAL; [40]) ^{1}H-MRS sequence with water suppression (TR 3000 ms, TE 8.5 ms, Phase cycle Auto, 192 averages from the right PFC voxel) in each participant [41]. Water unsuppressed spectra (16 averages) were also acquired. Six outer volume suppression slabs were applied (one on each side at 5mm from the edge of the cubic voxel) to suppress signals originating from outside the volume of interest and to minimize motion-related image-selected in vivo spectroscopy subtraction artifacts. Spectra were analysed using LCModel 6.3-1L with the basis set consisting of 19 simulated basis spectra; alanine (Ala), ascorbate (Asc), aspartate (Asp), creatine (Cr), γ-aminobutyric acid (GABA), glucose (Glc), glutamine (Gln), glutamate (Glu), glycine (Gly), glutathione (GSH), glycerophosphocholine (GPC) phosphocholine (PCh), lactate (Lac), myo-inositol (mI), N-acetylaspartate (NAA), N-acetylaspartateglutamate (NAAG), phosphorylethanolamine (PE), scyllo-inositol (Scyllo) & taurine (Tau).

The basis set was simulated using FID-A [42], for TE = 8.5 ms, magnetic field strength = 3 T and assuming ideal RF pulses. We excluded spectra with Cramer-Rao lower bounds (CRLB)

> 20% as reported by LCModel. In addition to metabolite levels, line widths and signal-to-noise ratios were estimated by LCModel. All spectra had a Line Width < 8 Hz and an SNR > 40 [41].

Metabolite levels have been shown to depend on the amount of cerebral spinal fluid (CSF), gray (GMV) and white matter (WMV) within the voxel [43], and inter-individual differences in cortical gray matter [44]. Correlations between PFC Glutamate and GMV and WMV WMV are reported in the supplementary material. To account for these potential confounds we used the T1-weighted anatomical images to estimate the gray and white matter content of the right PFC voxel in which the ^{1}H-MRS measures were performed using GABA Analysis Toolkit (Gannet 2.0, http://gabamrs.blogspot.co.uk/) adapted to work with Siemens SPECIAL data. The segmentation was performed using "new segment" in SPM 8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). CSF, GMV and WMV were then accounted for in the expression of Glu and GABA levels using LCModel [45, 46]; corrected metabolite levels are referred to as *Glu Corr* and *GABA Corr* using the formula *Glu Corr = (Glu*(43300*GMV+ 35880*WMV+55556*CSF))/(35880*(1-CSF))* and *GABA Corr = (GABA*(43300*GMV+ 35880*WMV+55556*CSF))/(35880*(1-CSF)).*

Additionally, because previous studies investigating the relationship between Glu and BOLD signal during cognitive control have used metabolite ratios relative to the synchronously-acquired Cr signal [20, 47] we report Glu/Cr results in a supplementary analysis (Supplementary Materials). Differences between LTA and HTA groups in right mPFC metabolite levels, as well as SNR, Line Width and CRLB were established using independent sample t-tests. Additionally, we calculated the BF_{10} for each comparison to assess the likelihood of the model relative to the null hypothesis. As no a-priori hypotheses for other 1H-MRS metabolite levels were stated, statistical tests for GABA, NAA, Myo-inositol, Choline and Creatine by trait anxiety group are reported in Supplementary Material.

2.6.fMRI data analysis

Functional MRI data were analysed using the Statistical Parametric Mapping software package (SPM12, Welcome Department of Cognitive Neurology, London, UK, www.fil.ion.ucls.ac.uk/spm/spm12). The anatomical and Echo Planer images (EPI) were reoriented manually based on the anterior commissure - posterior commissure axis. The images were corrected for slice timing. Motion correction was performed for functional images using six movement parameters to reduce motion artefacts. Volumes were co-registered to the high-resolution T1-weighted image and normalized into the Montreal Neurological Institute (MNI) template using parameters generated by unified segmentation of the T1-weighted structural image. The transformed data were smoothed using an 8 mm full width at half maximum (FWHM) isotropic Gaussian kernel. A high-pass filter with a cut-off of 128 s was applied to reduce low-frequency noise.

A fixed effects general linear model (GLM) was used to model data from the Stroop task at the 1st level based on event related Congruent and Incongruent colour-word trials. The number of error trials were modelled as regressors of no interest and Fixation cross trials were modelled implicitly. The six motion correction parameters were included as regressors of no interest in 1st level models. Contrast images were created for each participant at the 1st level to examine the main effect of condition (Congruent vs. Incongruent). The contrast Incongruent > Congruent was specified for each 1st level model to establish the effect of interference on whole brain activity at the single subject level.

These 1st level contrasts were then entered into a second-level ANCOVA to examine the

main effect of task (Incongruent > Congruent trials). To assess the effect of trait anxiety on DLPFC activation we entered 1st level contrast images into a regression model in SPM v12 as power was insufficient to detect small to medium effects using an independent samples t-test (See Supplementary Material).

These 1st level contrasts were entered into a second-level ANCOVA with each participants trait anxiety group (LTA vs. HTA) and PFC Glu Corr levels to examine task related activation during incongruent trials (Incongruent > Congruent), the effect of trait anxiety group on task related activation and the interaction effect for group x Glu Corr levels. Furthermore each participant's mean ER were included as a covariate of no interest to control for the effects of task performance on brain activation as these different between LTA and HTA groups. As the effect of group on estimated IQ scores was non-significant we chose not to include estimated IQ as a covariate in ANCOVA.

Because of our a-priori hypothesis that trait anxiety would specifically be associated with increased activity in DLPFC regions during a task requiring cognitive control we used a region of interest (ROI) approach (x, y, z = +/-34, 36, 24, small volume correction (SMV) sphere = 12mm). The DLPFC ROI was based on previous reviews of fMRI tasks that manipulate cognitive control [48, 49] and a previous study which reports a positive correlation between trait anxiety and DLPFC activity during a high load condition [5]. As effects of anxiety have been reported in left [5], right [9, 10, 50] and bilateral DLPFC activity [11, 51] we chose to test for effects in a bilateral DLPFC ROI. Exploratory full brain analyses are reported in Supplementary Materials. For all analyses ER were included as a covariate of no interest. Significance results are reported at a threshold of $p<.05$ (FWE-peak-level). To represent results graphically parameter estimates of activation were extracted from the peak voxel in analyses. No secondary analyses were performed on the extracted values [52, 53]. Plotting served the purpose of disentangling the effect revealed in the GLM.

3. RESULTS

3.1.Trait anxiety groups

A median-split based on STAI trait scores (median = 42) was used to establish low trait anxious (LTA) and high trait anxious groups. LTA and HTA groups differed significantly on STAI trait and state anxiety scores but not in age, or estimated IQ scores. There were no significant group differences between groups in alcohol consumption or cannabis use (See Table 1).

TABLE 1 HERE

3.2.Task Performance

Error rates: Participants' ER and RT during the Stroop task are shown in Figure 1. ANOVA revealed a significant effect of condition for ER ($F(1, 37) = 24.89$, $p < .001$, $\eta_{part}^2 = .40$) with a greater ER during incongruent trials across all participants. There was also a significant effect of trait anxiety group on ER ($F(1, 37) = 4.63$, $p = .038$, $\eta_{part}^2 = .11$) and significant group x task condition interaction effect ($F(1, 37) = 7.59$, $p = .009$, $\eta_{part}^2 = .17$) revealing that ER were greater in the incongruent condition for the HTA group.

Reaction Times: The main effect of condition on RT was non-significant ($F(1,37) = 1.84$, $p = .183$, $\eta_{part}^2 = .05$), however there was a significant effect of trait anxiety group on RT ($F(1, 37) = 4.54$, $p = .040$, $\eta_{part}^2 = .11$). Across the task the HTA group were slower than the LTA group. The group x task condition interaction was non- significant ($F(1, 37) = 0.13$, $p = .717$, $\eta_{part}^2 < .01$). The relative likelihood of this model compared to the null hypothesis is $BF_{10} = 0.29$.

FIGURE 1 HERE

3.3.fMRI: Stroop Effect

Compared to Congruent trials, Incongruent trials were associated with activation in the bilateral medial superior frontal gyrus and anterior cingulate cortex, the bilateral precentral gyrus extending to the right middle frontal, and in the left middle frontal and inferior gyrus and putamen (see Figure 2 and Table s2). There was no significant activation in the opposite contrast (Congruent > Incongruent trials) at a FWE corrected level of $p < .05$.

FIGURE 2 HERE

3.4.Effect of trait anxiety on DLPFC activity during incongruent trials

The effect of trait anxiety (STAI trait scores) on DLPFC activation was non-significant in bilateral DLPFC ROI during Incongruent > Congruent trials.

3.5.^{1}H- MRS: Glu Corr and DLPFC activation

PFC Glu Corr metabolite levels and spectra quality control data for LTA and HTA groups are reported in Table 2. All other metabolite levels are reported in Table s3. Differences between LTA and HTA groups for right PFC Glu Corr were non-significant (relative likelihood of this model compared to the null hypothesis $BF_{10} = 0.64$). The correlation between Trait anxiety scores and PFC Glu Corr levels was also non-significant ($r = .25$ $p = .121$).

TABLE 2 HERE

There was a significant interaction between PFC Glu Corr levels and trait anxiety group in the left DLPFC ROI (x, y, z = -26, 30, 18, Z = 3.60; $P_{FWE\ (Peak\text{-}level)} = .044$) (Figure 3C). The scatter plot in Figure 3A shows that during incongruent trials (Incongruent > Congruent) the LTA group showed a positive association between PFC Glu Corr levels and brain activity in the left middle frontal gyrus.

In the HTA group, during incongruent trials, PFC Glu Corr levels were not associated with activation in the DLPFC ROI. This interaction effect was not accounted for by task performance (ER).

FIGURE 3 HERE

4. DISCUSSION

The aim of this study was to examine the relationship between trait anxiety, DLPFC activation during a cognitive control task, and PFC Glu levels. Overall, participants performed the Stroop task with a high level of accuracy. As expected, during the Stroop task, error rates were greater during incongruent trials although unusually, reaction times did not differ significantly between congruent and incongruent conditions. It is unclear why this reaction time pattern was observed but it may have been due to a speed accuracy trade-off, trial/task pacing [54], or because the version of the task used in the present study used a single handed four-finger response system accounting for the relatively high reaction times observed in both congruent and incongruent task conditions. However, relative to the LTA group, the HTA group had greater ER during incongruent trials and were generally slower across the task. Reduced task performance (i.e. increased ER and RT) in the HTA group is consistent with the prediction that high levels of trait anxiety reduce *performance effectiveness* [6]. Reduced performance effectiveness during the incongruent trial condition of the Stroop task has been reported previously in anxious individuals [9, 55] and may be related to the high cognitive control requirements of the task.

During the Stroop task, fMRI data showed that incongruent (> congruent) trials were associated with activity in the anterior cingulate cortex (ACC) and medial superior frontal gyrus (supplementary motor area), the bilateral precentral gyrus, right middle frontal gyrus and left middle and inferior frontal gyri (as well as smaller activations in a number of subcortical regions). This finding is broadly consistent with previous fMRI studies/meta-analyses reporting functional activation during the Stroop task (e.g. [9, 56-58]). It is assumed that incongruent trials increase activity in ACC, supplementary motor area, and DLPFC regions due to the increased need for cognitive control.

In people with high trait anxiety, increased DLPFC activation without improved task performance effectiveness has been interpreted as reduced *processing efficiency* [9-11]. However, contrary to some previous fMRI findings, trait anxiety was not significantly associated with increased activation in the DLPFC during incongruent trials. Nevertheless, in the present study, the HTA group did demonstrate reduced performance effectiveness relative to the LTA group, suggesting that their DLPFC activation during incongruent trials may have been insufficient to perform the task effectively.

It has been reported previously that cortical Glu levels can predict anxiety levels [29] and that pharmacologically induced anxiety increases cortical Glu levels [30]. Examining our ^{1}H-MRS data however, there were no significant differences in PFC Glu levels between LTA and HTA groups. This may be due to our ^{1}H-MRS voxel placement, in the medial PFC, which differed from the ACC voxel placement used in these previous studies [20, 21]. We then examined how trait anxiety influenced the relationship between PFC Glu levels and DLPFC activation during cognitive control. We found a significant interaction between PFC Glu levels, trait anxiety and left DLPFC activation during incongruent task trials. This effect was driven by a positive association between PFC Glu levels and DLPFC activation in the LTA group, while PFC Glu and DLPFC activation were unrelated in HTA participants. This finding suggests a role for resting PFC Glu in DLPFC activation and is in line with previous studies by Falkenberg and colleagues [20] and Duncan and colleagues [47] that report resting Glu levels significantly influence how the brain implements cognitive control. Although speculative, resting PFC Glu may facilitate efficient processing during cognitive control through a higher capacity for energy turnover [59] and/or NMDAR function [19] that increase DLPFC activity in line with task demands. It should be made clear however, that the relationship between resting Glu concentrations and neural energy metabolism in humans is not fully understood [60, 61]. Thus, in the LTA group it is possible that such a positive

relationship between excitatory neurotransmission and task related activation in the DLPFC facilitates an effective and/or efficient neural processing mechanism when cognitive control is required. On the other hand, in the HTA group, no association between resting Glu levels and DLPFC activity was observed. This could be due to effects of trait anxiety on NMDAR function. Anxiety and neuroticism (a personality construct closely linked to trait anxiety) have been shown to affect NMDAR function [62, 63] and differences in NMDAR function can effect task-related interactions between default mode and FPN regions [19, 64]. The absence of this relationship between resting Glu levels and DLPFC activity in the HTA group may result in ineffective task performance; consistent with the predictions of ACT [6]. Together, these findings provide new insight into how a normally distributed personality dimension such as trait anxiety can affect the relationship between excitatory neurotransmission and activation in neural regions that support cognitive control. Future work could investigate if modulation of excitatory neurotransmission can ameliorate anxiety related effects on cognition.

4.1.Limitations

First, we report a number of null findings which raise issues regarding the power of the study. Our power calculations (Supplementary Material) suggest that the study was sufficiently powered to detect medium to large effect sizes (.50 - .90), that have been reported previously by studies investigating the associations between DLPFC activity [5], cortical glutamate levels [29] and trait anxiety. Clearly however, our study was not sufficiently powered to detect smaller effects sizes. This is important because previous studies examining the effects of trait anxiety on neurotransmitter levels for example have reported smaller effects sizes (e.g. [28]). Furthermore, Bayes Factors did not give a strong indication for either the null or the experimental hypothesis with regard to the relationship between trait anxiety and PFC Glu levels.

Thus, the null findings reported here need to be interpreted with some caution in as much as the study sample only provides sufficient power to detect larger effects. We cannot discount the possibility that the significant relationships between trait anxiety, DLPFC activity and/or cortical glutamate levels might be observed in a study powered to detect smaller effects sizes. Thus future studies aiming to examine the effect of trait anxiety on PFC Glu would need to recruit larger samples. It should also be noted that four of the 39 study participants were left-handed and laterality may affect stoop task performance [65].

Second, ^{1}H-MRS-fMRI analyses did not show any interaction effects within the right medial PFC voxel itself. Similar findings have been reported previously [20, 47], where no relationship between Glu and BOLD signal was seen in the measured region. This points to a more global effect of Glu on BOLD response, exerting 'long-range' influence on other regions via glutamatergic projection [20]. Notably this study relies on resting state Glu measurements rather than examining changes in these metabolite levels as a result of task demands. Though the use of resting-state MRS is common practice, PFC Glu levels differ between rest and task and reflect changes in other metabolic measures and cognitive demands [66]. Thus, future work could measure task-related differences in Glu levels to obtain a more accurate and dynamic insight into the neural basis of cognitive processes [67]; combined fMRI and MRS i.e. scan data collected simultaneously is a promising method to better understand the relationship between BOLD and neurotransmitter levels in the context of task processing [68].

Third, the concept of processing efficiency/inefficiency that is central to ACT does not tell us about the precise neural mechanisms that underlie the different patterns of brain activation in people with high levels of anxiety. For example, differences in intensity and timing of neural signalling (i.e. temporal dynamics) as well as resting cerebral blood flow and metabolism would be likely to affect activation in fMRI experiments [69]. However, we have shown here

that excitatory neurotransmission can modulate task related activation in the PFC and that this modulation effect is perturbed in people with high trait anxiety. Finally, there is emerging evidence that cognitive deficits in people with high trait anxiety/anxiety disorders are partly due to functional network imbalances (see [13]). Future work should examine how network interactions (i.e. FPN and Default Mode Network) are modulated by excitatory/inhibitory neurotransmission and how these interactions are affected by anxiety.

4.2.Conclusions

We have demonstrated that individual differences in trait anxiety affect the relationship between PFC Glu levels and DLPFC activation during cognitive control. This may contribute to ineffective task processing when cognitive control is required. These results need to be replicated in larger samples and more work is needed to examine how task related excitatory neurotransmission during cognitive control is affected by trait anxiety.

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REFERENCES

1. Gidron, Y., *Trait Anxiety*, in *Encyclopedia of Behavioural Medicine*, M. Gellman and R. Turner, Editors. 2013, Springer Science and Business Media: New York.
2. Sandi, C. and G. Richter-Levin, *From high anxiety trait to depression: a neurocognitive hypothesis.* Trends in Neurosciences, 2009. **32**(6): p. 312-320.
3. Heeren, A., E.E. Bernstein, and R.J. McNally, *Deconstructing trait anxiety: a network perspective.* Anxiety Stress Coping, 2018. **31**(3): p. 262-276.
4. Bishop, S., et al., *Prefrontal cortical function and anxiety: controlling attention to threat-related stimuli.* Nat Neurosci, 2004. **7**(2): p. 184-8.
5. Bishop, S.J., *Trait anxiety and impoverished prefrontal control of attention.* Nat Neurosci, 2009. **12**(1): p. 92-8.
6. Eysenck, M.W., et al., *Anxiety and cognitive performance: attentional control theory.* Emotion, 2007. **7**(2): p. 336-53.
7. Berggren, N. and N. Derakshan, *Attentional control deficits in trait anxiety: why you see them and why you don't.* Biol Psychol, 2013. **92**(3): p. 440-6.
8. Eysenck, M.W. and N. Derakshan, *New perspectives in attentional control theory.* Personality and Individual Differences, 2011. **50**(7): p. 955-960.
9. Basten, U., C. Stelzel, and C.J. Fiebach, *Trait anxiety modulates the neural efficiency of inhibitory control.* J Cogn Neurosci, 2011. **23**(10): p. 3132-45.
10. Basten, U., C. Stelzel, and C.J. Fiebach, *Trait anxiety and the neural efficiency of manipulation in working memory.* Cogn Affect Behav Neurosci, 2012. **12**(3): p. 571-88.
11. Fales, C.L., et al., *Anxiety and cognitive efficiency: differential modulation of transient and sustained neural activity during a working memory task.* Cogn Affect Behav Neurosci, 2008. **8**(3): p. 239-53.
12. Braver, T.S., et al., *Flexible neural mechanisms of cognitive control within human prefrontal cortex.* Proc Natl Acad Sci U S A, 2009. **106**(18): p. 7351-6.
13. Sylvester, C.M., et al., *Functional network dysfunction in anxiety and anxiety disorders.* Trends Neurosci, 2012. **35**(9): p. 527-35.
14. MacDonald, A.W., 3rd, et al., *Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control.* Science, 2000. **288**(5472): p. 1835-8.
15. Miller, E.K. and J.D. Cohen, *An integrative theory of prefrontal cortex function.* Annu Rev Neurosci, 2001. **24**: p. 167-202.
16. Brosnan, M.B. and I. Wiegand, *The Dorsolateral Prefrontal Cortex, a Dynamic Cortical Area to Enhance Top-Down Attentional Control.* J Neurosci, 2017. **37**(13): p. 3445-3446.
17. Jett, J.D., et al., *Deficits in cognitive flexibility induced by chronic unpredictable stress are associated with impaired glutamate neurotransmission in the rat medial prefrontal cortex.* Neuroscience, 2017. **346**: p. 284-297.
18. Nardecchia, F., et al., *Targeting mGlu5 Metabotropic Glutamate Receptors in the Treatment of Cognitive Dysfunction in a Mouse Model of Phenylketonuria.* Front Neurosci, 2018. **12**: p. 154.
19. Anticevic, A., et al., *NMDA receptor function in large-scale anticorrelated neural systems with implications for cognition and schizophrenia.* Proc Natl Acad Sci U S A, 2012. **109**(41): p. 16720-5.
20. Falkenberg, L.E., et al., *Resting-state glutamate level in the anterior cingulate predicts blood-oxygen level-dependent response to cognitive control.* Proc Natl Acad Sci U S A, 2012. **109**(13): p. 5069-73.
21. Javitt, D.C., *Glutamate as a therapeutic target in psychiatric disorders.* Molecular Psychiatry, 2004. **9**(11): p. 984-997.

22. van Wageningen, H., et al., *A 1H-MR spectroscopy study of changes in glutamate and glutamine (Glx) concentrations in frontal spectra after administration of memantine.* Cereb Cortex, 2010. **20**(4): p. 798-803.
23. Yucel, M., et al., *A combined spectroscopic and functional MRI investigation of the dorsal anterior cingulate region in opiate addiction.* Molecular Psychiatry, 2007. **12**(7): p. 691-702.
24. Kimbrell, T., et al., *Relationship of in vivo medial temporal lobe magnetic resonance spectroscopy to documented combat exposure in veterans with chronic posttraumatic stress disorder.* Psychiatry Research-Neuroimaging, 2005. **140**(1): p. 91-94.
25. Mahmutyazicioglu, K., et al., *Evaluation of the hippocampus and the anterior cingulate gyrus by proton MR spectroscopy in patients with post-traumatic stress disorder.* Diagn Interv Radiol, 2005. **11**(3): p. 125-9.
26. Whiteside, S.P., et al., *A magnetic resonance spectroscopy investigation of obsessive-compulsive disorder and anxiety.* Psychiatry Res, 2006. **146**(2): p. 137-47.
27. Dager, S.R., et al., *Two-dimensional proton echo-planar spectroscopic imaging of brain metabolic changes during lactate-induced panic.* Archives of General Psychiatry, 1999. **56**(1): p. 70-77.
28. Grachev, I.D. and A.V. Apkarian, *Anxiety in healthy humans is associated with orbital frontal chemistry.* Molecular Psychiatry, 2000. **5**(5): p. 482-488.
29. Modi, S., et al., *Glutamate level in anterior cingulate predicts anxiety in healthy humans: a magnetic resonance spectroscopy study.* Psychiatry Res, 2014. **224**(1): p. 34-41.
30. Zwanzger, P., et al., *Acute shift in glutamate concentrations following experimentally induced panic with cholecystokinin tetrapeptide--a 3T-MRS study in healthy subjects.* Neuropsychopharmacology, 2013. **38**(9): p. 1648-54.
31. Allen, P., et al., *Functional outcome in people at high risk for psychosis predicted by thalamic glutamate levels and prefronto-striatal activation.* Schizophr Bull, 2015. **41**(2): p. 429-39.
32. Fusar-Poli, P., et al., *Thalamic Glutamate Levels as a Predictor of Cortical Response During Executive Functioning in Subjects at High Risk for Psychosis.* Archives of General Psychiatry, 2011. **68**(9): p. 881-890.
33. Kompus, K., et al., *Resting-state glutamatergic neurotransmission is related to the peak latency of the auditory mismatch negativity (MMN) for duration deviants: An (1)H-MRS-EEG study.* Psychophysiology, 2015. **52**(9): p. 1131-9.
34. Stroop, J.R., *Studies of Interference in Serial Verbal Reactions (Reprinted from Journal Experimental-Psychology, Vol 18, Pg 643-662, 1935).* Journal of Experimental Psychology-General, 1992. **121**(1): p. 15-23.
35. Annett, M., *A classification of hand preference by association analysis.* Br J Psychol, 1970. **61**(3): p. 303-21.
36. Jastak, J. and G.S. Wilkinson, *Wide Range Achievement Test: Revised Edition*, J. Association, Editor. 1984: Wilmington.
37. Spielberger, C.D., et al., *Manual for the State-Trait Anxiety Inverntory*, C.P. Press, Editor. 1983: Palo Alto, CA.
38. Krabbendam, L., et al., *Using the Stroop task to investigate the neural correlates of symptom change in schizophrenia.* Br J Psychiatry, 2009. **194**(4): p. 373-4.
39. Jeffreys, H., *Theory of Probability*. 1939, Oxford: Clarendon.
40. Mlynarik, V., et al., *Localized short-echo-time proton MR spectroscopy with full signal-intensity acquisition.* Magn Reson Med, 2006. **56**(5): p. 965-70.
41. Godlewska, B.R., J. Near, and P.J. Cowen, *Neurochemistry of major depression: a study using magnetic resonance spectroscopy.* Psychopharmacology (Berl), 2015. **232**(3): p. 501-7.
42. Simpson, R., et al., *Advanced processing and simulation of MRS data using the FID appliance (FID-A)-An open source, MATLAB-based toolkit.* Magn Reson Med, 2017. **77**(1): p. 23-33.
43. Srinivasan, R., et al., *TE-averaged two-dimensional proton spectroscopic imaging of glutamate at 3 T.* Neuroimage, 2006. **30**(4): p. 1171-8.

44. Huster, R.J., et al., *Morphologic asymmetry of the human anterior cingulate cortex.* Neuroimage, 2007. **34**(3): p. 888-95.
45. Ernst, T., R. Kreis, and B.D. Ross, *Absolute Quantitation of Water and Metabolites in the Human Brain .1. Compartments and Water.* Journal of Magnetic Resonance Series B, 1993. **102**(1): p. 1-8.
46. Gasparovic, C., et al., *Use of tissue water as a concentration reference for proton spectroscopic imaging.* Magnetic Resonance in Medicine, 2006. **55**(6): p. 1219-1226.
47. Duncan, N.W., et al., *Involvement of glutamate in rest-stimulus interaction between perigenual and supragenual anterior cingulate cortex: a combined fMRI-MRS study.* Hum Brain Mapp, 2011. **32**(12): p. 2172-82.
48. Duncan, J. and A.M. Owen, *Common regions of the human frontal lobe recruited by diverse cognitive demands.* Trends Neurosci, 2000. **23**(10): p. 475-83.
49. Owen, A.M., *The functional organization of working memory processes within human lateral frontal cortex: the contribution of functional neuroimaging.* Eur J Neurosci, 1997. **9**(7): p. 1329-39.
50. Telzer, E.H., et al., *Relationship between trait anxiety, prefrontal cortex, and attention bias to angry faces in children and adolescents.* Biol Psychol, 2008. **79**(2): p. 216-22.
51. Karch, S., et al., *Influence of trait anxiety on inhibitory control in alcohol-dependent patients: simultaneous acquisition of ERPs and BOLD responses.* J Psychiatr Res, 2008. **42**(9): p. 734-45.
52. Poldrack, R.A. and J.A. Mumford, *Independence in ROI analysis: where is the voodoo?* Soc Cogn Affect Neurosci, 2009. **4**(2): p. 208-13.
53. Vul, E., et al., *Puzzlingly High Correlations in fMRI Studies of Emotion, Personality, and Social Cognition.* Perspect Psychol Sci, 2009. **4**(3): p. 274-90.
54. Renaud, P. and J.P. Blondin, *The stress of Stroop performance: physiological and emotional responses to color-word interference, task pacing, and pacing speed.* Int J Psychophysiol, 1997. **27**(2): p. 87-97.
55. Hopko, D.R., M.K. Hunt, and M.E.A. Armento, *Attentional task aptitude and performance anxiety.* International Journal of Stress Management, 2005. **12**(4): p. 20.
56. Derrfuss, J., et al., *Involvement of the inferior frontal junction in cognitive control: meta-analyses of switching and Stroop studies.* Hum Brain Mapp, 2005. **25**(1): p. 22-34.
57. Minzenberg, M.J., et al., *Meta-analysis of 41 functional neuroimaging studies of executive function in schizophrenia.* Arch Gen Psychiatry, 2009. **66**(8): p. 811-22.
58. van Veen, V. and C.S. Carter, *Separating semantic conflict and response conflict in the Stroop task: a functional MRI study.* Neuroimage, 2005. **27**(3): p. 497-504.
59. Rothman, D.L., et al., *In vivo NMR studies of the glutamate neurotransmitter flux and neuroenergetics: Implications for brain function.* Annual Review of Physiology, 2003. **65**: p. 401-427.
60. Boumezbeur, F., et al., *The Contribution of Blood Lactate to Brain Energy Metabolism in Humans Measured by Dynamic C-13 Nuclear Magnetic Resonance Spectroscopy.* Journal of Neuroscience, 2010. **30**(42): p. 13983-13991.
61. Mangia, S., et al., *Sustained neuronal activation raises oxidative metabolism to a new steady-state level: evidence from H-1 NMR spectroscopy in the human visual cortex.* Journal of Cerebral Blood Flow and Metabolism, 2007. **27**(5): p. 1055-1063.
62. Garakani, A., S.J. Mathew, and D.S. Charney, *Neurobiology of anxiety disorders and implications for treatment.* Mount Sinai Journal of Medicine, 2006. **73**(7): p. 941-949.
63. Aragam, N., et al., *TMPRSS9 and GRIN2B Are Associated with Neuroticism: a Genome-Wide Association Study in a European Sample.* Journal of Molecular Neuroscience, 2013. **50**(2): p. 250-256.
64. Montague, P.R., et al., *Role of NO production in NMDA receptor-mediated neurotransmitter release in cerebral cortex.* Science, 1994. **263**(5149): p. 973-7.

65. Christman, S.D., *Individual differences in stroop and local-global processing: a possible role of interhemispheric interaction.* Brain Cogn, 2001. **45**(1): p. 97-118.
66. Woodcock, E.A., et al., *Working Memory Modulates Glutamate Levels in the Dorsolateral Prefrontal Cortex during (1)H fMRS.* Front Psychiatry, 2018. **9**: p. 66.
67. Stanley, J.A. and N. Raz, *Functional Magnetic Resonance Spectroscopy: The "New" MRS for Cognitive Neuroscience and Psychiatry Research.* Front Psychiatry, 2018. **9**: p. 76.
68. Ip, I.B., et al., *Combined fMRI-MRS acquires simultaneous glutamate and BOLD-fMRI signals in the human brain.* Neuroimage, 2017. **155**: p. 113-119.
69. Poldrack, R.A., *Is "efficiency" a useful concept in cognitive neuroscience?* Dev Cogn Neurosci, 2015. **11**: p. 12-7.
70. Stone, J.M., et al., *Glutamate dysfunction in people with prodromal symptoms of psychosis: relationship to gray matter volume.* Biol Psychiatry, 2009. **66**(6): p. 533-9.

TABLES AND FIGURES

Table 1. *STAI scores, age, estimated IQ, alcohol and cannabis consumption for LTA and HTA groups.*

	LTA (n = 19)	HTA (n = 20)	Analysis
STAI trait	33.05 (5.05)	49.20 (9.33)	$t(37) = -6.67$, $p < .001$
STAI state	27.79 (5.41)	38.79 (10.76)	$t(37) = -4.83$, $p < .001$
Age (years)	22.31 (5.09)	21.80 (4.25)	$t(37) = .34$, $p = .73$
Estimated IQ	109.00 (9.91)	109.30 (10.80)	$t(37) = .01$, $p = .93$
Cannabis use (Moderate)	2	0	U = 155, $p = .27$
Alcohol use (Regular)	3	1	U = 183, $p = .78$

Table 2. *Means, Standard deviations and statistical analysis/Bayes Factors for ^{1}H-MRS quality control measures, right medial PFC Glu and GABA levels (Corr & /C) by LTA and HTA groups. Metabolite levels are represented in arbitrary units.*

PFC Glu/Met				Analysis (LTA vs. HTA)	
	LTA	HTA	Total	t-test result	BF_{10}
Glu Corr	7.41 (.58)	7.80 (1.10)	7.61 (.90)	t(37) = -1.36, p = .183	0.64
Glu/Cr	1.00 (.06)	1.05 (.08)	1.02 (.08)	t(37) = -1.99, p = .054, $\eta part^2 = .097$	1.44
S:N Ratio	60.00 (4.77)	60.85 (7.01)	60.44 (5.96)	t(37) = -.44, p = .662	0.34
Line Width in Hz	3.53 (.79)	4.26 (1.30)	3.90 (1.13)	t(31.67) = -2.128, p = .041, $\eta part^2 = .107$	1.71
Glu CRLB	4.05 (.62)	3.85 (.67)	3.95 (.65)	t(37) = .98, p = .335	0.46

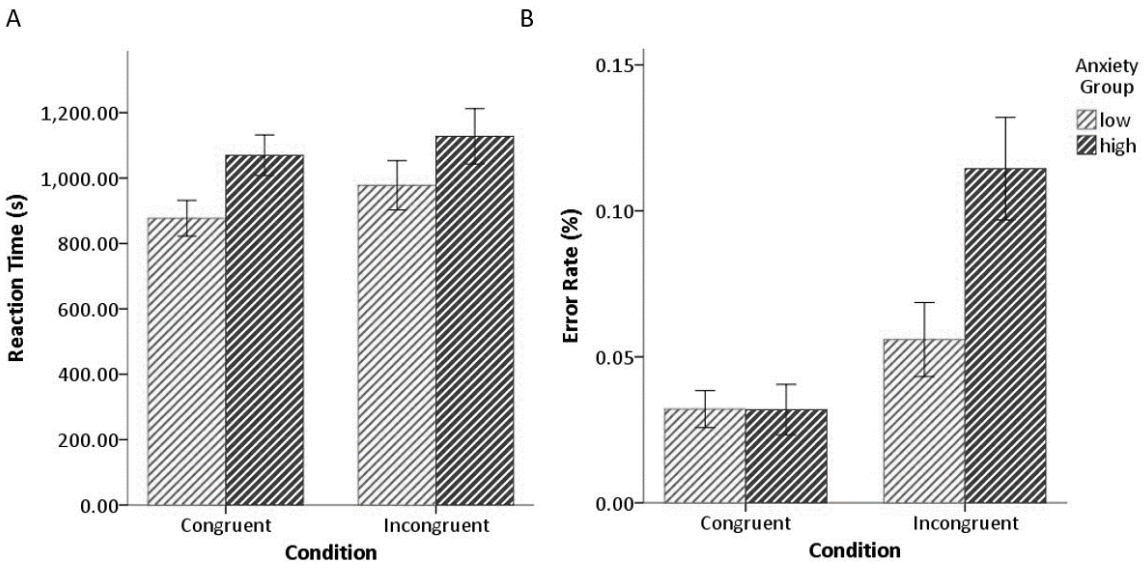

Figure 1. *Reaction time and error rate data for Stroop task. (A) Mean reaction time (RT) in milliseconds (ms) and (B) error rate (ER) % errors by trait anxiety group and task condition. Error bars show the standard error of the mean.*

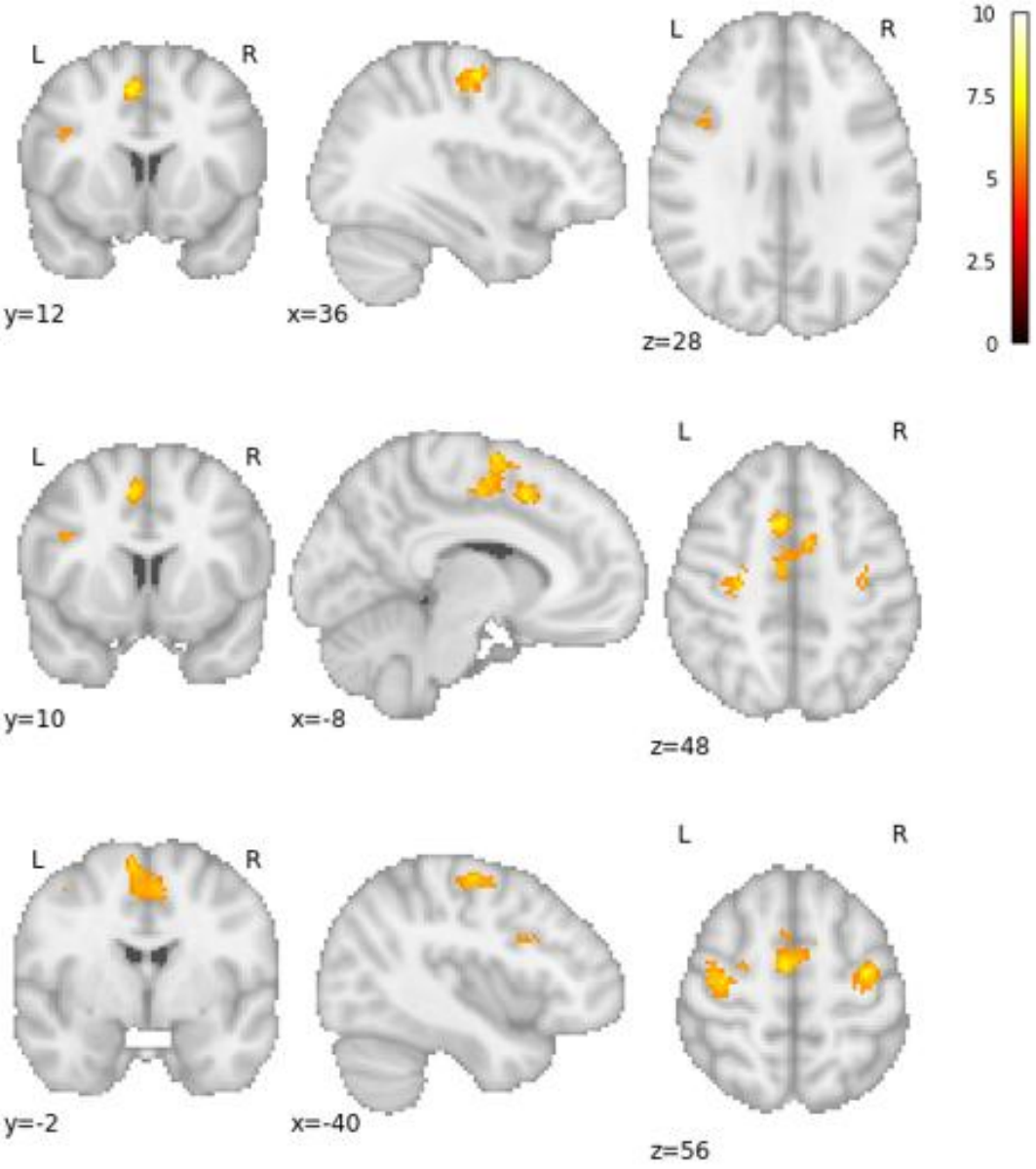

Figure 2. *(A) Statistical Parametric Maps in axial, coronal and sagittal sections showing the main effect of the Stroop task (incongruent > congruent) in cortical regions. Results displayed at p < .05 FWE peak corrected. The left side of the brain is on the left side of the image.*

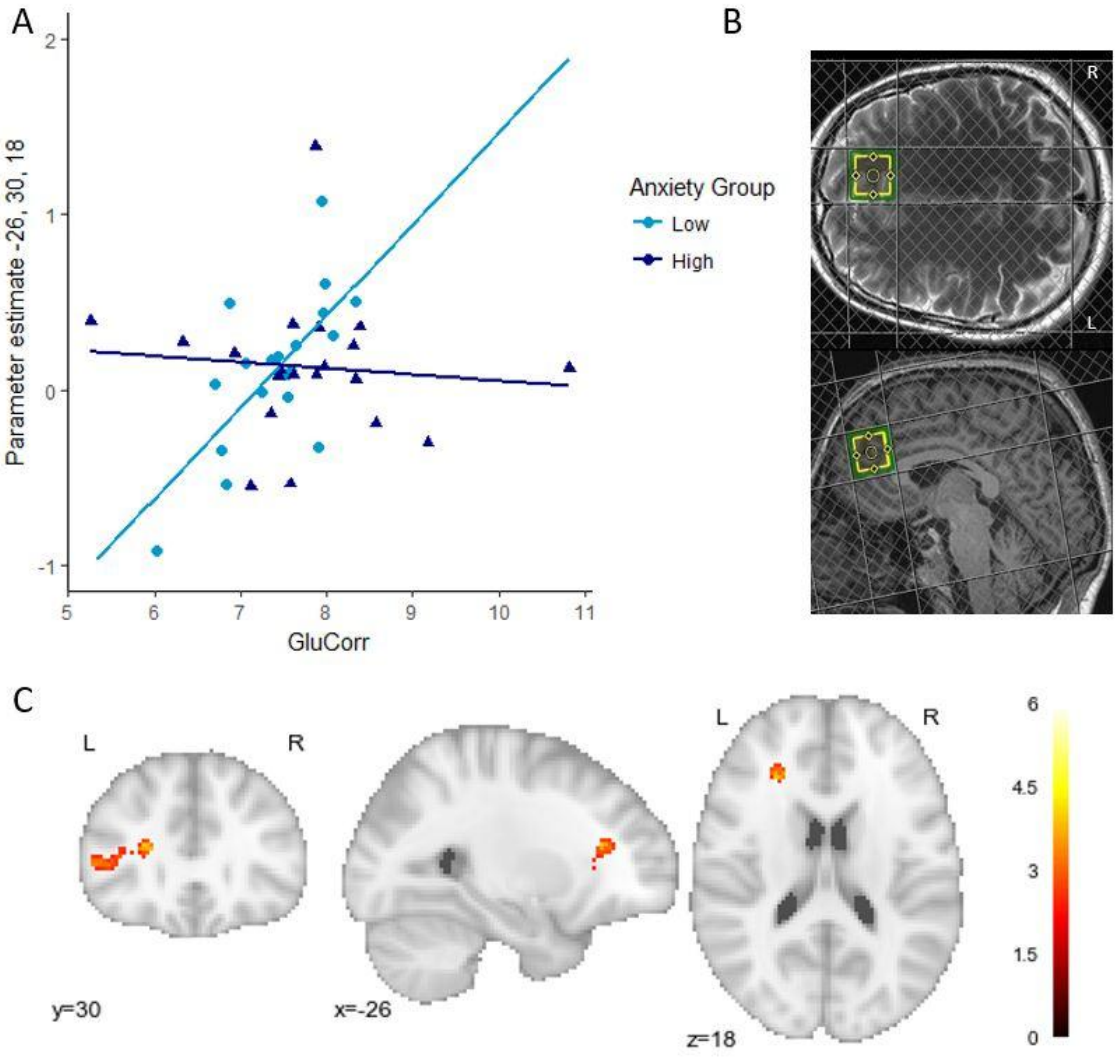

Figure 3. (A) Scatter plot and line of best fit showing individual contrast parameter estimates by right PFC Glu Corr levels (arb. unit) by trait anxiety group. (B) Positioning of the voxel for right medial PFC voxel for ^{1}H-MRS acquisition. (C) Statistical Parametric Map showing brain activations for trait anxiety Group x PFC Glu Corr interaction during incongruent trials at P =.05 FWE corrected threshold. Results displayed at p > .005 uncorrected for illustrative purposes.

SUPPLEMENTARY MATERIAL

METHODS

Power Calculations

Our power calculations suggest that, with independent group sizes of n = 19 (LTA) & 20 (HTA), we would only have sufficient power to detect a significant group difference (using an independent sample t-test) in DLPFC activity if the effect size was > .8 (large). Thus, our sample size is insufficient to detect small and medium effect sizes. However, based on the effect size of 0.49 reported by Bishop [5] for a significant positive correlation between STAI trait anxiety scores and DLPFC activity (see [5] Figure 2c), our power calculation show that an n = 36 has > 90% power to detect a significant positive association between STAI scores and DLPFC activation at p =.05 (one-tailed). As our n = 39 for this analysis, we can assume sufficient power.

To our knowledge only one previous study has reported differences in prefrontal cortical Glu levels (in the anterior cingulate cortex) for high vs. low trait anxiety groups [29]. This study reports an effect size of .85, but is based on a small sample. Generically, using mean and standard deviation data from an independent ^{1}H-MRS glutamate dataset [70] we calculated an effect size for PFC glutamate levels based on a small to medium (15%) change in glutamate levels between groups (Cohen's d = .90). Using an intermediate effect size of .875 our power calculation shows that n = 19 has >85% power to detect a significant independent group difference for PFC glutamate levels at p = .05 (one-tailed).

RESULTS

Task Performance with trait anxiety as a continuous covariate

A repeated measures ANCOVA including STAI trait scores as a continuous covariate, revealed no significant effect of condition on ER ($F(1, 37) = 1.08$, $p = .305$), there was however a significant effect of trait anxiety on ER ($F(1, 37) = 7.01$, $p = .012$, $\eta_{part}^2 = .16$). There was also a significant trait anxiety x task interaction effect ($F(1, 37) = 5.68$, $p = .022$, $\eta_{part}^2 = .13$). The relative likelihood of this model compared to the null hypothesis is $BF_{10} = 6410.85$.

The main effect of condition on RT was not significant ($F(1, 37) = 0.12$, $p = .730$), neither was there a significant effect of trait anxiety on RT ($F(1, 37) < 0.01$, $p = .993$). There was a trend towards a significant trait anxiety x task interaction effect ($F(1, 37) = 3.16$, $p = .084$, $\eta_{part}^2 = .08$). The relative likelihood of this model compared to the null hypothesis is $BF_{10} = 0.55$.

^{1}H- MRS: Glu Corr, trait anxiety (continuous variable) and DLPFC activation

In an additional analysis STAI trait anxiety scores were included as a continuous variable (covariate) in an otherwise identical analysis to what has been reported in the main results section. Within the DLPFC ROI there were no suprathreshold effects of trait anxiety during Incongruent > Congruent trials. There were furthermore no suprathreshold effects of Glu Corr. There was no significant interaction between Glu Corr and trait anxiety in the left DLPFC ROI but there was a significant interaction in the right DLPFC ROI (x, y, z = 24, 32, 22, $Z = 3.59$; $P_{FWE\ (Peak\text{-}level)} = .045$).

The whole brain analysis further showed a significant interaction between PFC Glu/Cr levels, trait anxiety and activity in the right anterior cingulate gyrus (x, y, z = 14, 24, 36, Z = 4.83; $P_{FWE\ (Peak\text{-}level)}$ = .034).

Trait Anxiety Group x ^{1}H-MRS interactions exploratory whole brain analysis

The whole brain analysis revealed no regions with a significant interaction effect for PFC Glu Corr levels × trait anxiety group during incongruent trials.

^{1}H- MRS: Glu/Cr

There were no significant correlations between PFC GMV and Glu levels (r = .20 p =.21, BF_{10} = 0.41), nor between WMV and Glu levels (r = -.24 p = .14, BF_{10} = 0.57). Thus, it is unlikely that individual differences in PFC GMV and WMV influence Glu levels. There were no significant differences between LTA and HTA groups for PFC Glu/Cr. However, there was a strong trend towards higher PFC Glu/Cr levels in the HTA group relative to the LTA group (t(37) = -1.99, p = .054, η_{part}^2 = .097, BF_{10} = 1.44; Table 2).

Trait Anxiety Group x ^{1}H-MRS interactions for Glu/ Cr

There was a trend towards an interaction between PFC Glu/Cr levels, trait anxiety group and activity in the right DLPFC ROI (x, y, z = 30, 28, 16, Z = 3.45; $P_{FWE\ (Peak\text{-}level)}$ = .070). There was a trend towards a positive association between right PFC Glu/Cr and DLPFC brain activity in the LTA group. The whole brain analysis further showed a significant interaction between PFC Glu/Cr levels, trait anxiety group and activity in the left inferior/middle frontal gyrus (x, y, z = -48, 26, 10, Z = 4.83; $P_{FWE\ (Peak\text{-}level)}$ = .036) (Figure s1B). The scatter plot in Figure s1A shows that during incongruent trials (incongruent > congruent) the LTA group showed a positive association between right PFC Glu/Cr and brain activity in the left inferior/middle frontal gyrus. In the HTA group, during incongruent trials, PFC Glu/Cr levels

were not associated with activation in this region. This interaction effect was not accounted for by task performance (ER).

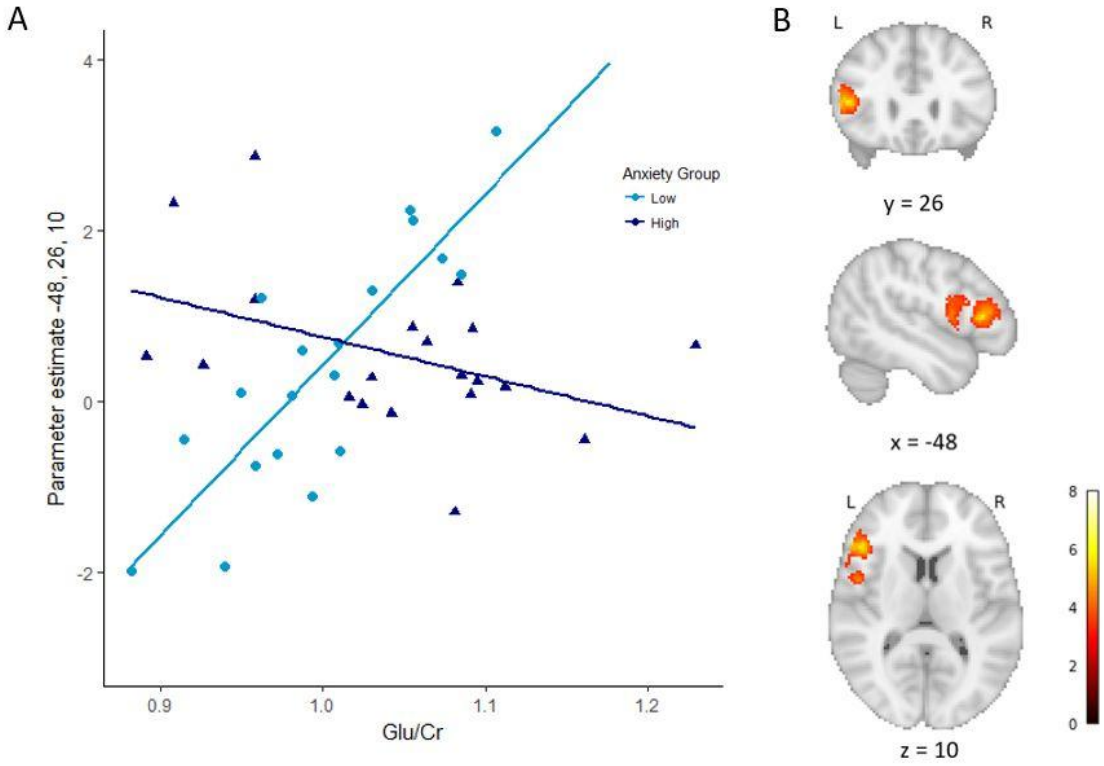

Figure s1. *(A) Scatter plot and line of best fit showing individual contrast parameter estimates by PFC Glu/Cr levels (arb. unit) by trait anxiety group. Statistical Parametric Map showing brain activations for trait anxiety Group x PFC Glu/Cr interaction during incongruent trials. Results displayed at p>.001 uncorrected for illustrative purposes.*

SUPPLEMENTARY TABLES

Table s1*: Frequency of alcohol and cannabis consumption across participants.*

	No or experimental use	Occasional use	Moderate use	Regular and severe use
Alcohol	4	21	12	2
Cannabis	35	2	2	0

Table s2: *Regions and MNI coordinates for activations during Incongruent > Congruent Stroop Trials ($p_{FWE\ peak} < .05$).*

Cluster	Hemisphere	P_{FWE} (Peak-level)	Z	MNI coordinates (mm)		
				x	y	z
Anterior cingulate gyrus/superior frontal gyrus	R	<.001	5.87	-8	12	48
		<.001	5.77	-6	-8	52
		0.001	5.55	-8	-4	66
Precentral gyrus	R	<.001	5.70	36	-12	56
		0.024	4.91	32	-20	50
Precentral gyrus	L	0.001	5.55	-36	-16	56
		0.001	5.53	-28	-18	48
		0.006	5.23	-26	-10	52
Anterior cingulate gyrus	R	0.008	5.17	16	16	34
		0.022	4.93	10	16	40
Inferior Frontal Gyrus/ Precentral Gyrus/ Middle frontal Gyrus	L	0.021	4.95	-40	10	28
Precentral Gyrus/ Middle frontal Gyrus	L	0.025	4.90	-38	0	40
Insula	L	0.026	4.90	-42	22	0
Posterior Supramarginal Gyrus	L	0.035	4.83	-54	-46	22
Precentral Gyrus/ Middle frontal Gyrus	L	0.041	4.79	-36	-2	44
Middle Frontal Gyrus/ Inferior Frontal Gyrus	L	0.042	4.78	-38	18	28
Putamen	L	0.049	4.74	-24	0	14

Table s3

Table s3: *Mean, standard deviations and statistics for metabolite levels for all participants, LTA and HTA groups (metabolite levels reported in arbitrary units).*

PFC Metabolite Total Levels		LTA Group	HTA Group	Analysis (LTA vs. HTA group)
Creatine	6.47 (.52)	6.57 (.44)	6.37 (.59)	t(37) = 1.17 p = .249
GABA	1.78 (.28)	1.73 (.22)	.1.83 (.33)	t(37) = 1.06 p = .296
GABA Corr	2.06 (.35)	1.97 (.27)	2.14 (.40)	t(37) = -1.62, p = .113
GABA/Cr	.28 (.05)	.26 (.03)	.29 (.06)	t(37) = -1.65, p = .107
Gln	.20 (.06)	.19 (.05)	.21 (.07)	t(37) = -.93 p =.360
Glu	6.59 (.58)	6.54 (.46)	6.64 (.68)	t(37) = -.52 p =.609
mI	5.67 (.47)	5.73 (.50)	5.62 (.44)	t(37) =.699 p =.489
NAA	8.39 (.66)	8.50 (.44)	8.29(.81)	t(37) = 1.02 p = .315

Credit Autor Statement

Elenor Morgenroth: Writing – Review & Editing, Investigation, Visualization, Formal Analysis, Writing – Original Draft

Natasza Orlov: Writing – Review & Editing, Formal Analysis

David J Lythgoe: Writing – Review & Editing, Formal Analysis

James M Stone: Writing – Review & Editing

Holly Barker: Writing – Review & Editing, Investigation

James Munro: Writing – Review & Editing, Investigation

Michael Eysenck: Writing – Review & Editing

Paul Allen: Conceptualization, Supervision, Writing – Review & Editing, Project Administration, Funding Acquisition, Formal Analysis, Writing – Original Draft

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